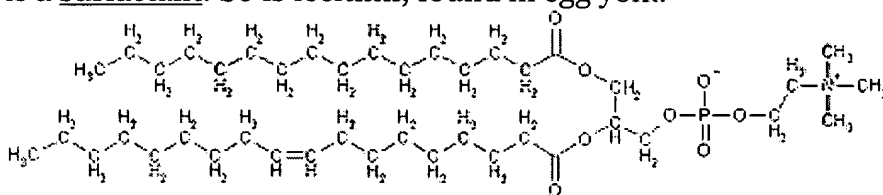


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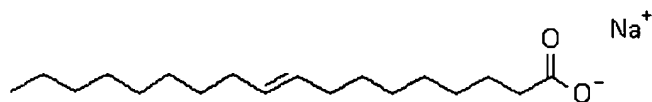
Emulsifier

A compound added to a mixture of two immiscible liquids in order to make it an emulsion, and not just two layers of liquid lying on top of each other. An emulsifier will usually be a molecule where one end is highly soluble in water and the other is highly soluble in oil. Sodium dodecyl sulfate, the active ingredient in bubble-blowing mixture, is a surfactant. So is lecithin, found in egg yolk:

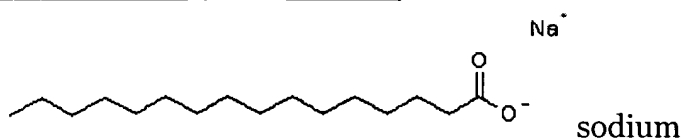


Soap

A soap is a type of surfactant that is derived from the saponification reaction (hydrolysis) of a vegetable oil. A soap has a carboxylate group on the end which can form a complex with calcium ions in hard water. (This causes soaps to form precipitates giving rise to a "soap scum".) Soaps are often called fatty acid salts. Common soaps are:

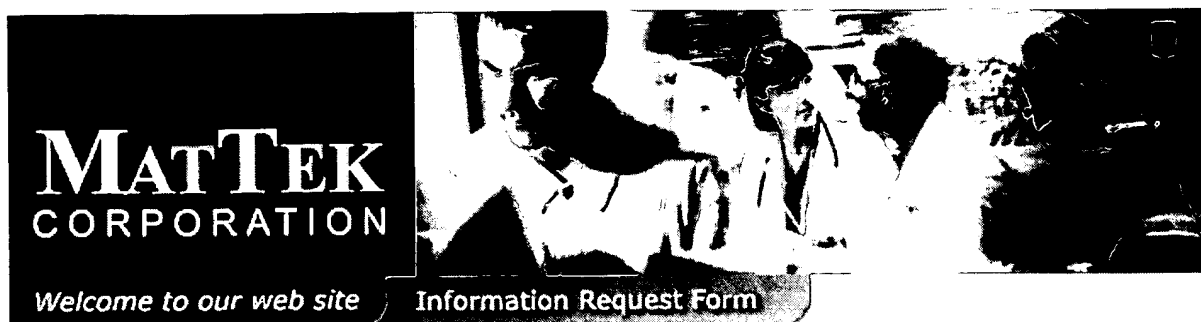


sodium oleate (from olive oil) and



Surfactant

From SURFace ACTive AgeNT. A substance which prefers to exist at the boundary between two other substances - for example, detergents have one end highly soluble in greasy, non-polar substances and one end soluble in water. Sodium dodecyl sulfate is a common surfactant. See also emulsifier.



338.

ASSESSMENT OF SKIN ABSORPTION AND METABOLISM OF ARACHIDONIC ACID & GLYCERYL ARACHIDONATE USING IN VITRO DIFFUSION CELL

TECHNIQUES. Eppler^{1,2}, A.R., Kraeling¹, M.E.K., Wickett², R.L., Bronaugh¹, R.L.

¹Office of Cosmetics and Colors/ Cosmetic Toxicology Branch, US Food & Drug Administration, Laurel, MD, ²College of Pharmacy, University of Cincinnati, Cincinnati, OH. Poster Presented at the Society of Toxicology Annual Meeting, Baltimore, MD. March 21-25, (2004).

Keywords: AA, Anti-oxidant, Arachidonic acid (AA), Cosmetic Ingredient Review, Dermal absorption, Dermal irritation, Diffusion cells, EPI-606X, Edema, Eicosatetraenoic acid, Emollient, Emulsifying agent, Epiderm, FDA Voluntary Cosmetic Reporting Program, Glyceryl arachidonate (GA), In vitro percutaneous absorption, Linoleic acid, Linolenic acid, Metabolism, Moisturizing preparations, Percutaneous absorption, Percutaneous absorption/Penetration studies, Proinflammatory mediators, Shampoos, Skin conditioning, Stratum corneum, Surfactant, Topical application

Endpoints: Metabolism, Permeability

Materials Tested: AA, Arachidonic acid (AA), GA, Glyceryl arachidonate (GA)

Summary: Arachidonic acid (AA), has been used in cosmetics as a surfactant-cleansing and emulsifying agent. Glyceryl arachidonate (GA), a skin conditioning agent and emollient, may be partially metabolized by ester hydrolysis in skin to AA. Based on the Cosmetic Ingredient Review (CIR) Panel's concern that there was a lack of dermal absorption data for AA, in vitro percutaneous absorption and metabolism studies were initiated. To simulate normal consumer use, AA and GA were applied in an oil in water emulsion (2mg/cm²) to skin samples 200µm thick in flow-through diffusion cells perfused with a physiological buffer. To assay for permeation, viable fuzzy rat and human skin were dosed with [¹⁴C] AA containing ~0.5µCi (0.01mg) AA /cell while [³H]GA was applied to viable and cadaver human skin at ~0.5µCi (0.003mg) GA/cell. For metabolism analysis, the skin equivalent Epiderm was dosed with [³H]GA under similar conditions. Receptor fluid fractions were collected at 6h intervals over a 24h dosing period. Skin penetration was determined by liquid scintillation counting and expressed as a percent of the applied dose. High performance liquid chromatography was used to assess metabolism to AA. Absorption of AA through rat skin was 19.8 ± 5.3 % (mean ± SEM) compared to only 1.4 ± 0.3 % through human skin. Total AA penetration (receptor fluid plus skin levels) in rat and human skin was 52.3 ± 7.3 and 20.1 ± 5.4 %, respectively. Total GA penetration of viable skin was found to be 11.3 ± 2.1% with only 3.2 ± 0.5% absorbed through the skin. In cadaver skin, 4.8 ± 0.8% GA was absorbed through skin with a total penetration of 6.7 ± 1.2%. Assay of the Epiderm receptor fluid found ~50% absorption of radioactivity and 3.0 ± 2.1% GA conversion to AA. In conclusion, percutaneous absorption of AA was less through human than rat skin, while GA absorbed through Epiderm was metabolized to AA.

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☐ 1: *Int J Toxicol.* 2004;23 Suppl 2:55-94.

Final report of the amended safety assessment of Glyceryl Laurate, Glyceryl Laurate SE, Glyceryl Laurate/Oleate, Glyceryl Adipate, Glyceryl Alginate, Glyceryl Arachidate, Glyceryl Arachidonate, Glyceryl Behenate, Glyceryl Caprate, Glyceryl Caprylate, Glyceryl Caprylate/Caprate, Glyceryl Citrate/Lactate/Linoleate/Oleate, Glyceryl Cocoate, Glyceryl Collagenate, Glyceryl Erucate, Glyceryl Hydrogenated Rosinate, Glyceryl Hydrogenated Soyate, Glyceryl Hydroxystearate, Glyceryl Isopalmitate, Glyceryl Isostearate, Glyceryl Isostearate/Myristate, Glyceryl Isostearates, Glyceryl Lanolate, Glyceryl Linoleate, Glyceryl Linolenate, Glyceryl Montanate, Glyceryl Myristate, Glyceryl Isotridecanoate/Stearate/Adipate, Glyceryl Oleate SE, Glyceryl Oleate/Elaidate, Glyceryl Palmitate, Glyceryl Palmitate/Stearate, Glyceryl Palmitoleate, Glyceryl Pentadecanoate, Glyceryl Polyacrylate, Glyceryl Rosinate, Glyceryl Sesquioleate, Glyceryl/Sorbitol Oleate/Hydroxystearate, Glyceryl Stearate/Acetate, Glyceryl Stearate/Maleate, Glyceryl Tallowate, Glyceryl Thiopropionate, and Glyceryl Undecylenate.

[No authors listed]

The safety of 43 glyceryl monoesters listed as cosmetic ingredients was reviewed in a safety assessment completed in 2000. Additional safety test data pertaining to Glyceryl Rosinate and Glyceryl Hydrogenated Rosinate were received and served as the basis for this amended report. Glyceryl monoesters are used mostly as skin-conditioning agents--emollients and/or surfactant--emulsifying agents in cosmetics. The following 20 glyceryl monoesters are currently reported to be used in cosmetics: Glyceryl Laurate, Glyceryl Alginate, Glyceryl Arachidonate, Glyceryl Behenate, Glyceryl Caprylate, Glyceryl Caprylate/Caprate, Glyceryl Cocoate, Glyceryl Erucate, Glyceryl Hydroxystearate,

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Safety assessment of S: Acid, Butyloctyl Salicyla Calcium Salicylate, C12

Glyceryl Isostearate, Glyceryl Lanolate, Glyceryl Linoleate, Glyceryl Linolenate, Glyceryl Myristate, Glyceryl Oleate/Elaidate, Glyceryl Palmitate, Glyceryl Polyacrylate, Glyceryl Rosinate, Glyceryl Stearate/Acetate, and Glyceryl Undecylenate. Concentration of use data received from the cosmetics industry in 1999 indicate that Glyceryl Monoesters are used at concentrations up to 12% in cosmetic products. Glyceryl Monoesters are not pure monoesters, but are mostly mixtures with mono-, di-, and tri-esters. The purity of commercial and conventional Monoglyceride (Glyceryl Monoester) is a minimum of 90%. Glyceryl Monoesters (monoglycerides) are metabolized to free fatty acids and glycerol, both of which are available for the resynthesis of triglycerides. Glyceryl Laurate enhanced the penetration of drugs through cadaverous skin and hairless rat skin in vitro and has been described as having a wide spectrum of antimicrobial activity. A low-grade irritant response was observed following inhalation of an aerosol containing 10% Glyceryl Laurate by test animals. Glyceryl monoesters have little acute or short-term toxicity in animals, and no toxicity was noted following chronic administration of a mixture consisting mostly of glyceryl di- and mono- esters. Glyceryl Laurate did have strong hemolytic activity in an in vitro assay using sheep erythrocytes. Glyceryl Laurate, Glyceryl Isostearate, or Glyceryl Citrate/Lactate/Linoleate/Oleate were not classified as ocular irritants in rabbits. Undiluted glyceryl monoesters may produce minor skin irritation, especially in abraded skin, but in general these ingredients are not irritating at concentrations used in cosmetics. Glyceryl monoesters are not sensitizers, except that Glyceryl Rosinate and Hydrogenated Glyceryl Rosinate may contain residual rosin, which can cause allergic reactions. These ingredients are not photosensitizers. Glyceryl Citrate/Lactate/Linoleate/Oleate was not mutagenic in the Ames test system. Glyceryl Laurate exhibited antitumor activity and Glyceryl Stearate was negative in a tumor promotion assay. At concentrations higher than used in cosmetics, Glyceryl Laurate did cause moderate erythema in human repeat-insult patch test (RIPT) studies, but the other glyceryl monoesters tested failed to produce any significant positive reactions. Glyceryl Rosinate was irritating to animal skin at 50%, but did not produce sensitization in clinical tests at concentrations up to 10% and covered with semiocluded patches. There is reported use of Glyceryl Rosinate at 12% in mascara, which is somewhat higher than the concentration in the clinical testing. It was reasoned that the available data do support the safety of this use because there would be minimal contact with the skin and no occlusion. The safety of Arachidonic Acid was not documented and substantiated for cosmetic product use in an earlier safety assessment and those same

Alkyl Salicylate, Caprylic Salicylic Acid, Hexyldod Salicylate, Isocetyl Salicylate, Isodecyl Salicylate, Magnesium Salicylate, Potassium Salicylate, Ethylhexyl Salicylate, Potassium Salicylate, Methyl Salicylate, Myristyl Salicylate, Sodium Salicylate, TEA-Salicylate, Tridecyl Salicylate, Toxic

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safety questions apply to Glyceryl Arachidonate. Based on these data, the Cosmetic Ingredient Review (CIR) Expert Panel found that these glyceryl monoesters are safe as cosmetic ingredients in the present practices of use and concentration: except that the available data are insufficient to support the safety of Glyceryl Arachidonate. Additional data needed to support the safety of Glyceryl Arachidonate include (1) dermal absorption data; and, based on the results of the absorption studies, there may be a need for (2) immunomodulatory data; (3) carcinogenicity and photocarcinogenicity data; and (4) human irritation, sensitization, and photosensitization data.

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Links

Determination of surface tensions of proteins. II. Surface tension of serum albumin, altered at the protein-air interface.

Absolom DR, Van Oss CJ, Zingg W, Neumann AW.

Serum albumin, which itself has a surface tension of congruent to 70.3 erg/cm², when dissolved in water lowers the surface tension of water from 72.5 to congruent to 50 erg/cm², as measured by a variety of means, including the pendant drop, the Wilhelmy plate and the platinum ring methods. Equally low and even lower surface tensions are found with the contact angle method, on a thin layer of albumin that had been adsorbed onto a low energy surface and subsequently exposed to air. Surface tensions of drops of albumin solutions varying in concentration from 0.01 to 5.5% (w/v) yielded, with a contact angle method, values that only varied between 67 and 61 erg/cm². With the pendant drop, the Wilhelmy plate and the platinum ring methods, one essentially measures the surface tension at the air-liquid interface, at which proteins tend to adsorb, and where reversible or irreversible reorientation can be expected. The same holds for a thin layer of protein adsorbed onto a low energy surface, exposed to air. Thus, when through the very act of surface tension measurement, or after adsorbing protein onto a substrate, protein is exposed at the air-liquid interface, it apparently loses the pronounced hydrophilicity characteristic of its native hydrated state and manifests through reorientation a much more hydrophobic tertiary configuration.

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RESEARCH COMMUNICATION

Reduction of the surface-tension-lowering ability of surfactant after exposure to hypochlorous acid

T. Allen MERRITT,* John D. AMIRKHANIAN,* Harold HELBOCK,* Barry HALLIWELL†‡§ and Carroll E. CROSS†

Divisions of *Neonatology and †Pulmonary and Critical Care Medicine, University of California, Davis, Medical Center, Sacramento, CA 95817, U.S.A. and

‡Pharmacology Group, University of London, King's College, Chelsea Campus, Manresa Road, London SW3 6LX, U.K.

The reactive species hypochlorous acid (HOCl/OCl^-) is a major product of the respiratory burst in activated neutrophils. We studied the effects of HOCl/OCl^- on human surfactant and upon surfactants Survanta, KL_4 and Exosurf, utilizing a pulsating surfactometer for measuring surface tension. HOCl/OCl^- induced a marked dose-dependent decrease in the surface-tension-lowering activity of human surfactant. The surfactant containing surfactant proteins B and C (Survanta) was less sensitive; however, synthetic surfactants with or without peptides were not

affected by HOCl/OCl^- (KL_4 , Exosurf). Ascorbic acid and GSH protected human surfactant against inactivation by HOCl/OCl^- . We suggest that HOCl/OCl^- produced by activated phagocytes in the alveolar compartment of the lung could damage endogenous surfactant and affect the function of exogenously administered natural or other surfactants, especially if ascorbic acid and GSH levels in the lung lining fluids are subnormal, as is known to be the case in some inflammatory lung diseases.

INTRODUCTION

Surfactant is critical for maintaining low surface tensions in the alveoli, and the surfactant proteins A, B and C (SP-A, SP-B and SP-C) (especially the last two) play a critical role in this function [1,2]. Some reactive oxygen species, in addition to serum proteins, have been shown to diminish the surface-tension-lowering ability of surfactant [3,4], and this inhibition of function contributes to the severity of respiratory distress syndrome (RDS) in preterm infants [5]. Surfactant replacement therapy has yielded significant improvements in morbidity and mortality in premature infants with RDS [6] and, from preliminary studies, in full-term infants with severe respiratory failure [7]. More recently, surfactant treatment has been tested in patients with adult respiratory distress syndrome (ARDS) [8,9]. Surfactant replacement therapy has been performed with surfactant (containing SP-A, SP-B and SP-C) obtained from human amniotic fluid, with modified lipid-extracted surfactants of bovine or porcine origin containing SP-B and SP-C, and with totally synthetic preparations containing either synthetic peptides (such as KL_4 surfactant [10]) or without proteins at all (such as Exosurf), as described in Table 1.

In ARDS, increased numbers of phagocytes are recruited to the alveolar spaces [11], and there is evidence that they are activated to produce increased quantities of both proteases and reactive oxygen species, such as superoxide radical ($\text{O}_2^{\cdot-}$) and H_2O_2 [12,13]. In the presence of iron, these can be converted into more reactive species such as the hydroxyl radical, $\cdot\text{OH}$ [14]. It has already been shown that iron-dependent radical-generating systems damage surfactant surface-tension-lowering functions [3,4]. However, activated neutrophils produce large amounts of hypochlorous acid (HOCl/OCl^-), a reactive oxidizing species which can damage proteins by attacking thiol groups, methionine, tryptophan, and amino groups [15–17]. HOCl/OCl^- is generated by H_2O_2 -dependent oxidation of Cl^- ions by the neutrophil enzyme myeloperoxidase [18].

In this study, we show that HOCl/OCl^- damages the surface-tension-lowering ability of human surfactant. We have also examined the effect of HOCl/OCl^- upon a surfactant containing SP-B and SP-C, and upon synthetic surfactant preparations being used clinically (or undergoing clinical trials), as it is interesting to know if these various preparations are also susceptible to HOCl/OCl^- -induced oxidative damage.

MATERIALS AND METHODS

Sodium hypochlorite (NaOCl), 5,5'-dithiobis-(2-nitrobenzoic acid), reduced glutathione (GSH) and ascorbic acid were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Inorganic salts were from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Surfactant of human amniotic fluid origin (IND 26308) was prepared as previously reported [5]. Surfactant KL_4 was kindly supplied by Dr. Charles Cochrane at the Scripps Research Institute (La Jolla, CA, U.S.A.). Survanta and Exosurf were purchased from Ross Laboratories (Columbus, OH, U.S.A.) and Burroughs-Wellcome (Research Triangle Park, NC, U.S.A.) respectively.

Exposures to HOCl/OCl^-

The concentration of NaOCl was determined using the molar absorption coefficient at 290 nm of $350 \text{ M}^{-1} \cdot \text{cm}^{-1}$ at pH 12. Serial dilutions of a stock solution of 40 mM NaOCl were made in normal saline and the required concentrations dispensed into the suspension of surfactant.

Two concentrations of each surfactant were used for HOCl/OCl^- treatment, 2.5 and 5.0 mg of phospholipid/ml dispersed in a final volume of 0.5 ml, concentrations approximating to those found in the alveolar lining fluids. The control vials contained only normal saline with phosphate buffer. The following order of mixing was used: 50 μl of surfactant, 350 μl of normal saline, 50 μl of 0.5 M $\text{Na}_2\text{HPO}_4/\text{KH}_2\text{PO}_4$ at pH 7.4, 50 μl of HOCl/OCl^- (diluted previously in normal saline), to

Abbreviations used: SP-A, -B and -C, surfactant proteins A, B and C; RDS, respiratory distress syndrome; ARDS, adult RDS.

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Table 1 Compositions of surfactants used in the study

Surfactant type	Composition
Natural human surfactant (from amniotic fluid)	Lipid + SP-A, SP-B and SP-C
Survanta	Lipid + bovine SP-B and SP-C
KL ₄ (synthetic)	Lipid + leucine/lysine peptide model of SP-B
Exosurf	Lipid only

produce the required concentration of each component of the mixture in the final volume of 500 μ l. After each treatment, the pH of the mixture was measured to ensure that it remained at 7.4.

Surface tension measurements

Treated and control samples were incubated from 0.25 to 2 h at 37 °C before surface tension measurements were taken using a pulsating-bubble surfactometer (Electronetics Corporation, Amherst, NY, U.S.A.) as previously described [19]. Minimum surface tension in mN/m was recorded at 1, 2 and 3 min of inflation and deflation of a bubble at 20 cycles/min. The adsorption rate of surfactant to an air-liquid interface was measured through plots of surface tension versus time in seconds during formation of the bubble at maximum radius.

Effect of antioxidants

The effects of ascorbic acid and GSH were examined by mixing them, at the final concentrations stated, with surfactants before incubation with HOCl/OCl⁻.

RESULTS

At pH 7.4, HOCl is approx. 50 % ionized to hypochlorite anion, OCl⁻ [20]. Since we do not know whether HOCl, OCl⁻, or both of these species cause the damage described below, we have used the term HOCl/OCl⁻ throughout this paper.

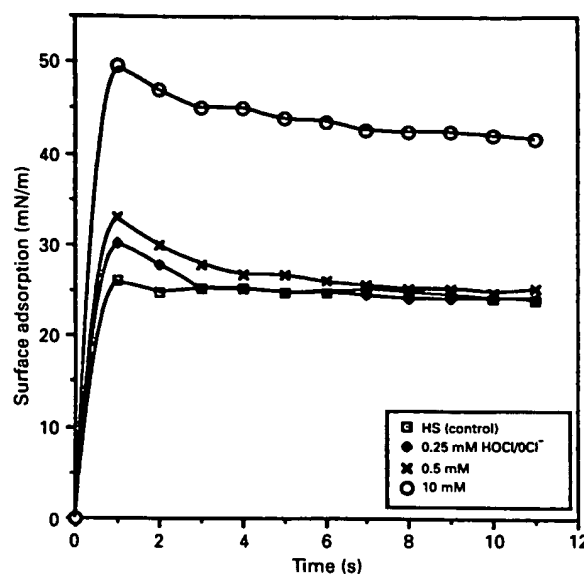
Effects of surface adsorption

As shown in Figure 1, the surface adsorption of human surfactant was minimally affected by HOCl/OCl⁻, except at 10 mM concentrations of HOCl/OCl⁻. There was a similar minimal effect on surface adsorption with the other surfactants (results not shown).

Effects on surface tension minimum (mN/m) of surfactants

Table 2 shows, in common with other observations [21,22], that human surfactant, at concentrations of 2.5 and 5.0 mg/ml, lowered surface tensions to 0.9 and 1.4 mN/m respectively, while KL₄ surfactant achieved values of 3.2 and 3.0 mN/m, Survanta 6.0 and 4.0 mN/m, and Exosurf 27 nN/m at both concentrations. Treatment with HOCl/OCl⁻ affected these preparations differently. As we have shown that the biophysical activity of these preparations is affected by pH [22,23], it was essential to ensure that the pH remained at 7.4 by careful buffering. The effect of HOCl/OCl⁻ was complete after incubation for 0.25 h, in keeping with the high reactivity of these species [24].

Human surfactant was strongly affected by HOCl/OCl⁻. Even a 125 μ M concentration significantly ($P < 0.01$) decreased its

**Figure 1** Surface adsorption measurements made after the formation of the bubble to maximum bubble diameter with human surfactant (2.5 mg/ml) with various concentrations of HOCl/OCl⁻

Surface adsorption at 10 s after bubble formation shows minimal change except at the highest concentration (10 mM) of HOCl/OCl⁻.

Table 2 Minimum surface tension (mN/m) of surfactants at concentrations of 2.5 mg/ml (A) and 5.0 mg/ml (B) after exposure to HOCl/OCl⁻

Inhibitory effects of HOCl/OCl⁻ on various surfactants at 2.5 mg/ml (A) and 5.0 mg/ml (B). Human surfactant shows the lowest surface tension, but is markedly affected by HOCl/OCl⁻. Exosurf containing no surfactant protein shows the highest baseline surface tension, but is unaffected by 2 mM HOCl/OCl⁻. Survanta and KL₄ have low surface tension as controls, but are also affected by HOCl/OCl⁻; however, KL₄ is affected only at a concentration of 2.5 mg/ml by 2.0 mM HOCl/OCl⁻. * $P < 0.0001$, † $P < 0.001$, †† $P < 0.01$ compared with the control value. Values are expressed as mean (S.E.M.) for $n = 6$.

HOCl/OCl ⁻ concn. (mM)	Minimum surface tension (mN/m)			
	Human surfactant	Survanta	KL ₄	Exosurf
(A) 2.5 mg/ml surfactant				
Control (0)	0.9 (0.9)	6.0 (0.5)	3.2 (0.4)	27 (0.3)
0.125	5.0 (0.2)††	7.0 (1.0)	3.5 (0.2)	28 (0.2)
0.250	8.0 (0.2)†	8.0 (1.0)	3.5 (0.3)	28 (0.1)
0.500	8.8 (0.7)†	9.0 (0.3)†	4.2 (0.2)	27 (0.5)
1.000	14.8 (1.1)*	17.7 (0.7)*	4.0 (0.2)	28 (0.2)
2.000	20.5 (0.2)*	20.0 (1.0)*	7.0 (0.2)††	27 (0.2)
(B) 5.0 mg/ml surfactant				
Control (0)	1.4 (0.4)	4.0 (0.4)	3.0 (0.4)	27 (0.25)
0.125	5.0 (0.8)††	7.4 (0.5)	3.8 (0.1)	27 (0.1)
0.250	7.9 (0.5)†	7.5 (0.6)†	3.6 (0.1)	27 (0.2)
0.500	14.0 (2.0)*	10.0 (0.3)*	3.3 (0.1)	27 (0.2)
1.000	18.0 (0.4)*	15.0 (0.7)*	3.5 (0.4)	28 (0.1)
2.000	19.0 (0.3)*	19.3 (0.4)*	5.8 (0.8)††	28 (0.1)

surface-tension-lowering ability (Table 2). By contrast, Survanta was only affected by HOCl/OCl⁻ at concentrations ≥ 0.5 mM and KL₄ surfactant only at concentrations ≥ 2 mM. Exosurf was not affected by even 2 mM HOCl/OCl⁻.

Table 3 Protective effect of GSH on HOCl/OCl⁻-induced inhibition of surface-tension-lowering ability of human surfactant

At 0.5 mM HOCl/OCl⁻, 0.2 and 0.5 mM GSH has a significant protective effect on HOCl/OCl⁻-induced inhibition of surface-tension-lowering capability, whereas at 1.0 mM HOCl/OCl⁻ 1.0 mM GSH was required to prevent HOCl/OCl⁻ inhibition. **P* < 0.0001 compared with the control value, (ANOVA). Values are expressed as means (S.E.M.), *n* = 6.

HOCl/OCl ⁻ concn. (mM)	Surface tension (mN/m)			
	— GSH	+ 0.2 mM GSH	+ 0.5 mM GSH	+ 1 mM GSH
Control	3.4 (0.4)	4.0 (0.2)	3.4 (0.4)	3.5 (0.1)
0.5	14.0 (0.2)*	6.2 (0.7)	5.8 (0.8)	—
1.0	18.0 (0.4)*	11.5 (1.4)*	6.7 (0.7)	5.8 (0.8)

Table 4 Protective effect of ascorbic acid (Asc) on HOCl/OCl⁻-induced inhibition of surface-tension-lowering ability of human surfactant

At 1.0 mM HOCl/OCl⁻ 0.5 mM ascorbic acid and 0.2 mM ascorbic acid has significant concentration-dependent effects on the HOCl/OCl⁻-induced inhibition of surface-tension-lowering capability. **P* < 0.0001 compared with the control value, (ANOVA). Values are expressed as means (S.E.M.), *n* = 6.

HOCl/OCl ⁻ concn. (mM)	Surface tension (mN/m)			
	— Asc	+ 0.2 mM Asc	+ 0.5 mM Asc	+ 2 mM Asc
Control	3.5 (0.4)	4.3 (0.2)	—	4.3 (0.2)
1.0	18.0 (0.4)*	10.0 (0.7)*	7.3 (0.7)	5.7 (0.8)

Effects of antioxidants

GSH and ascorbic acid are important antioxidants in broncho-alveolar lavage fluids [25–27], and their ability to scavenge HOCl/OCl⁻ has been reported [24,28]. Table 3 shows that GSH was able to protect human surfactant against damage by HOCl/OCl⁻. For example, 0.5 mM GSH protected significantly against the effects of 1 mM HOCl/OCl⁻. Table 4 shows that ascorbic acid was similarly protective at concentrations of 0.5 mM.

DISCUSSION

Surfactant is known to be susceptible to oxidative damage by activated neutrophils, but previous studies have concentrated upon iron-ion-dependent reactions [3,4]. However, in the present study we show that HOCl/OCl⁻, a major product generated by activated neutrophils, can rapidly destroy the surface-tension-lowering ability of surfactant. Inhibition was complete after 0.25 h (the shortest time in which measurements could be made), but given the reactivity of HOCl/OCl⁻, it is probable that damage was much quicker than this [24]. It has been reported that 5×10^6 activated neutrophils generate 88.3 ± 23.6 nmol of HOCl/OCl⁻ per ml in 2 h, i.e. about 100 μ M [29]. Although HOCl/OCl⁻ is very unlikely to accumulate 'free' *in vivo*, its effective concentration at localized sites of formation in the alveolar compartment may be much greater than this. Hence, the observation that 125 μ M HOCl/OCl⁻ damages the human surfactant surface-tension-lowering ability is not without physiological relevance, especially as large numbers of neutrophils are found in the airway fluid of neonates with RDS [30] and adults with ARDS [11].

Survanta was less susceptible to inhibition by HOCl/OCl⁻,

and KL₄ and Exosurf were nearly unaffected. This perhaps suggests that any effects of HOCl/OCl⁻ on the lipid component of surfactant are not the major factor(s) responsible for its surface-tension-lowering ability. However, different lipid compositions and conformations of proteins (where present) in each preparation could affect such tentative conclusions [22]. Our results suggest that surfactant preparations such as KL₄ surfactant or Exosurf may have the advantage of being less susceptible to oxidative damage, although the surface-tension-lowering capacity of Exosurf on the pulsating bubble and in animal models of RDS may be inferior to natural surfactants [31].

GSH is reported to be present in alveolar lining fluids at concentrations approaching 200 μ M and ascorbate at even higher concentrations [26,27]. Hence, they could be protective against HOCl/OCl⁻ generated *in vivo*. In several models of inflammatory lung diseases, it has been shown that GSH levels are diminished [32], so that damage to surfactant by HOCl/OCl⁻ is feasible. Such damage would be additional, and probably much faster, than that produced by proteases and by other reactive oxygen species, such as H₂O₂ and OH⁻.

While our studies show that exposure of human surfactant or Survanta to HOCl/OCl⁻ *in vitro* results in inhibition of surface tension, the precise molecular mechanisms involved have not been established. HOCl/OCl⁻ has been shown to produce chlorohydrins from unsaturated fatty acids [33], and three (human, Survanta and KL₄) of the four surfactants utilized in the current studies contain unsaturated fatty acids. Exosurf, containing no unsaturated fatty acid, was least affected by HOCl/OCl⁻. However, it seems more likely that critical essential amino acid residues which are important for interaction with lipid constituents (possibly thiol and amino groups) on SP-A, SP-B and SP-C are being damaged by HOCl/OCl⁻. This could contribute to the particular sensitivity of human surfactant surface activity to be inhibited by HOCl/OCl⁻, for it represents the only surfactant studied to contain the major surfactant protein, SP-A. This speculation is buttressed by findings that SP-A is a particular target of attack by ozone [34] and by nitrogen dioxide [35], two other strong oxidants.

In summary, our results demonstrate a significant loss of surface-tension-lowering ability of human lung surfactant after exposure to an oxidizing agent known to be produced in large amounts by activated phagocytes *in vivo* [18]. In developing strategies for surfactant administration in clinical conditions of surfactant deficiency or surfactant dysfunction [7,10] and where alveolar inflammation can be expected to exist, it would appear useful to consider including selected antioxidants (such as GSH and ascorbic acid) in the administered therapeutic surfactant mixtures.

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